アフリカ産ナマズClarias gariepinus仔稚魚の耳石の発達と耳石日周輪

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Otolith Development and Daily Increment Formation in African catfish *Clarias gariepinus* (Burchell, 1815) (Pisces: Clariidae) Larvae and Juveniles

Shinsuke MORIOKA*1 and Seiji MATUMOTO*1

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Abstract: Features of the sagitta, asteriscus and lapillus of laboratory-hatched African catfish *Clarias gariepinus* larvae and juveniles were investigated until 34 days after hatching. The sagittae were round-shaped until 6 days after hatching, then, became arrowhead-shaped with development of rostra. Increments in the sagittae were observable until bases of rostra, but invisible in rostra. The asteriscus appeared on 4-5 days after hatching with oval-shape, having an ambiguous core. As fish grow, notches developed around its margin. The lapilli were round-shaped until 6 days after hatching, then, became oval-shaped in which notches on posterior margin appeared 22 days after hatching. Several pre-hatch increments were present, and subdaily increments were frequently observed in the lapilli. But, increments in the lapilli were distinctive from the core to the margin, and increased mostly at the rate of one per day after hatching. These features indicate the lapillus being the most appropriate for daily increments analysis in this species.

Key words: *Clarias gariepinus*; Otoliths development; Daily increment validation; Lapillus utility

African Catfish *Clarias gariepinus* (Burchell) widely distributes over the African continent, and is the one of important commercial fish species in Lake Malawi, Malawi. This species is known to have an omnivorous feeding habit and grows fast1,2). Aquaculture of this species is, thus, widely applied in a number of countries in Africa. In such background, researches on the aquaculture of this species, e.g. seed production, rearing techniques and nutritional aspects have been made so far1,2). However, recent decline in catch of *C. gariepinus* in the country3,4) leads to necessity to elucidate the biology of this species relevant to resource management, apart from its importance in development of aquaculture.

In order to make researches into fish biology, information on the early life history is indispensable to assess growth, survival and recruitment success of the species. For examining fish early life, otolith daily increment analysis has been broadly applied and developed since Pannella5). Several important cyprinids species in Malawi have been preliminary studied using otolith increments analysis6,7,8). On *Clarias gariepinus*, however, otolith information has not been obtained so far, although several analyses on the adult population of this species have been made9,10).

To determine the age of fish in days, the suitable otolith is needed to be identified and the formation pattern of otolith increments should be validated if they are deposited on a daily basis. This study, therefore, aimed to describe the morphological features of otoliths and to detect the most suitable otolith for aging, and to validate the increment formation pattern using laboratory-hatched specimens.

Materials and methods

A total of fifty-eight larvae and juveniles of

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Clarias gariepinus were used in this study. Fish were obtained from artificially fertilized eggs (dry method) in Aquaculture and Fisheries Science Department of Bunda College, University of Malawi. Eggs hatched on 15 March 2001 (day 0) and ca. 20,000 fish were stocked in 0.5 t plastic tank. Zooplankton (rotifers and copepods) and nauplii of Artemia sp. were given once a day at the density of 5–10 per ml from day 2 until day 8. Thereafter, 10,000 fish were stocked in an earthen pond (200 m², 50 cm deep) which has been fertilized by chicken manure for enhancing the occurrence of natural food organisms before stocking. Fish fed mainly on natural diet (zooplankton, insect juveniles, juveniles of snails) in the pond. Ten fish were collected on 3, 6, 9, 11, 22 and 34 days after hatching, and were immediately preserved in 70% ethanol. Water temperature was 21–25ºC. Although photoperiod was not artificially controlled, dark and light periods were approximately 12 h and 12 h, respectively.

The sagittae, lapilli, and asterisci were extracted after measuring total length (TL, mm), and were mounted in epoxy resin on glass slides. When otoliths were opaque, they were ground with sand paper (# 1500) and lapping films (3, 6 and 9 μm mesh). Ground surfaces of otoliths were occasionally etched with 0.1 N hydrochloric acid to emphasize the contrast of increments. Otolith increments were observed under an optic microscope with transmitted light (x 200–1000), and maximum radii (μm) of each otolith were measured.

**Results**

Total length of fish at hatching was 4.07 ± 0.11 mm (mean ± SD, n = 10) and reached 84.95 ± 13.72 mm at 34 days after hatching (n = 10, Fig. 1). Fish used in this study were in juvenile stage on 22 days after hatching based on appearance of skeleton components, while were not on 11 days after hatching yet.

At 3 days after hatching, the asterisci were absent, while the sagittae and lapilli were present. The sagittae and lapilli were round-shaped with an obvious core (Fig. 2-A-a and 2-B-a). After 6 days, the asterisci existed being oval-shaped with an ambiguous core (Fig. 2-C-a). This indicated that the asterisci appeared between 4 and 5 days after hatching. The sagittae started elongating anterior- and posterior-ward with forming rostra as being arrowhead-shaped, while the lapilli were round-shaped (Fig. 2-A-b and 2-B-a). The lapillus started elongating anterior- and posterior-ward at 9 days to become oval (Fig. 2-B-b). Rostra of the sagittae, thereafter,
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Further elongated as being thin plates (Fig. 2-A-c). At 22 days after hatching, notches were observed on posterior margin of the lapillus (Fig. 2-B-c) and periphery of the asteriscus (Fig. 2-C-b). Relationships between total length (mm) and maximum radii (µm) of each otolith were expressed with allometric regressions before 11 days after hatching and linear regressions after 11 days after hatching (Table 1, Fig. 3).

Around the core of the lapillus, the distinctive check was observed, the diameter of that check being 12.66 ± 1.57 µm (mean ± SD, n = 31) (Fig. 4). This check was considered a hatch check, inside which several increments were observed as pre-hatch increments (Fig. 4). Increment counts of the lapillus outside a hatch check tended to increase at the rate of one increment per day as expressed by the following regression; N = 0.99 · D + 0.04 (r = 0.99, n = 57) (Figs. 5 and 6). The slope of the formula did not significantly differ from 1 (P > 0.05, comparison of regression slopes), and mean of increment counts on each day did not differ significantly from actual age in days (t-test, P > 0.05). These indicated the lapillus increments being formed on a daily basis and suitable for daily increment analysis until 34 days after hatching. Subdaily increments were frequently deposited throughout the development of the lapillus (Fig. 7A). However, structures of subdaily increments were vague and could be eliminated under

### Table 1. Regressions between total length (mm) and maximum radii (µm) of each otolith before and after 11 days after hatching. R and L are maximum radii and total length, respectively

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<th>Otolith</th>
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<td>Sagitta</td>
<td>(R = 0.58 \cdot L^{2.99} ) (r = 0.99, n = 39)</td>
<td>(R = 7.02 \cdot L + 50.33 ) (r = 0.99, n = 15)</td>
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<tr>
<td>Lapillus</td>
<td>(R = 1.39 \cdot L^{1.66} ) (r = 0.98, n = 39)</td>
<td>(R = 11.48 \cdot L - 28.39 ) (r = 0.97, n = 28)</td>
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<td>Asteriscus</td>
<td>(R = 0.57 \cdot L^{3.91} ) (r = 0.86, n = 29)</td>
<td>(R = 4.63 \cdot L + 41.49 ) (r = 0.98, n = 24)</td>
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**Fig. 3.** Relationships between total length of fish and maximum radii of the sagitta (top), lapillus (middle) and asteriscus (bottom).

**Fig. 4.** Otolith core of the lapillus in *Clarias gariepinus* larvae (14.05 mm TL). C: core of the lapillus. Arrow and bar indicate a hatch check and 20 µm, respectively. Pre-hatch increments are deposited inside a hatch check.
microscopic observation by arranging the focal distance (Fig. 7B).
In the sagitta and asteriscus, increments were also observed (Fig. 8A and B). However, rostra of the sagitta were fragile and frequently destroyed during extracting procedure. The asteriscus has an ambiguous core leading to difficulty in discerning the first increment, and it appeared later than the lapilli after hatching (between 4 and 5 days after hatching). These features demonstrated that the sagittae and

Fig. 5. Relationship between days after hatching and increment counts in the lapillus.

Fig. 6. The lapillus of *Clarias gariepinus* larvae at age of 9 days after hatching (13.65 mm TL). C: core of the lapillus. Bar indicates 50 μm. Dots indicate daily increments.

Fig. 7. Photos under different focusing of microscope in same portion of the lapillus. A: subdaily increments, B: daily increments under arranged focal distance. Bars indicate 50 μm. Dots in B indicate daily increments.

Fig. 8. A: the sagitta of *Clarias gariepinus* larvae (9 days after hatching, 13.65 mm TL), B: the asteriscus (6 days, 10.05 mm TL). Bars indicate 100 μm.
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Discussion

Growth patterns of otoliths differ before and after metamorphosis in many species\(^{11}\). Metamorphosis of this species in this study took place during 11 – 22 days after hatching, and growth patterns of otoliths are considered to change during metamorphosis (Fig. 3).

Rosen and Greenwood\(^{12}\) reported that the sagittae of ostariophysi elongate and produce the fragile rostrum structures inappropriate for increments analysis\(^{13}\). This agrees with the observation of the sagitta in this study in *C. gariepinus* (Fig. 8) as one of ostariophysi. Similar observations were reported in cyprinids as in ostariophysi, e.g., *Delitistes luxatus*, *Chasmistes brevirostris*\(^{13}\), *Ptychocheilus lucius*\(^{14}\), *Engraulicypris sardella*, *Opisaridium microcephalum*\(^{7}\) and *Opisaridium microlepis*\(^{8}\).

The asteriscus is also elucidated inappropriate because of its ambiguous core (Fig. 8) and delayed appearance. Delayed appearance of the asteriscus is also observed in several fish species\(^{13,15,16}\).

In contrast, the lapillus showed visible increments from the core to the margin throughout its development and was appropriate for otolith increments analysis in this species as well as reported in several other cyprinids\(^{7,8,13,14}\). Increments in the lapillus were formed on a daily basis after hatching, although several pre-hatch increments occurred inside a hatch check. This shows the importance in discerning the hatch check for age-unknown specimens from the natural population. In addition, subdaily increments were frequently deposited in the lapillus, although they could be eliminated by arranging the focal distance of microscope. Morioka and Machinandiarena\(^{17}\) succeeded the identification of real daily increments by using poorly focused microscopic observation in the sagittae of *Genypterus blacodes*\(^{17}\). Nishimura\(^{18}\) suggested that subdaily increments occurred during fast-growing phase in *Theragra chalcogramma*. Neison and Geen\(^{19}\) reported that the multiple feeding per day caused multiple increments in *Onchorhynchus tshawytscha*. Fish used in this study are considered to undergo the diet-rich environment both in the laboratory and the earthen pond, and to grow fast. This environment may cause the deposition of subdaily increments observed in this study.

Better utility of the lapillus than the sagitta for daily increment analysis was also reported in non-ostariophysi, e.g. *Hirundichthys affinis* of Beloniformes\(^{16}\), *Genypterus blacodes* of Ophidiformes\(^{17}\). The lapillus, therefore, would be appropriate for daily increments analysis in most species, and its further utilization is recommended not only in ostariophysi but in others.

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アフリカ産ナマズ Clarias gariepinus 仔稚魚の耳石の発達と耳石周囲

森岡伸介・松本誠司

マラウィ産ナマズ Clarias gariepinus 仔稚魚の扁平石、星状石、磔石の成長に伴う形態変化と耳石微細構造の形成時期を調査した。本種の扁平石は明瞭な核を有し、孵化直後は円形で、成長に伴い体軸方向に突起（rostrum）が伸長した。星状石の核周辺では輪紋は明瞭に観察されるが、両端の突起内部では観察が困難であった。また、この突起部は魚体からの採取や研磨の際に割れ易いことから、扁平石は日齢検定に適さないと判断された。星状石は孵化後4～5日目の間に形成され、不明瞭な核を有することがから、日齢形質としては不適と判断された。一方、磔石は明瞭な核を持ち、その周辺に鱗化輪が形成され、鱗化輪の内側には微細な輪紋が観察された。鱗化輪の外側に形成された輪紋数と鱗化後日数間係は傾きが1であり、1日1本の割合で輪紋が形成されたことが明らかになった。この結果から、磔石の輪紋は日齢周で、3種の耳石のうち最も日齢検定に適しているものと結論された。磔石には多くのsubdaily incrementsが認められたが、それらの間隔は日齢よりも狭く、顕微鏡の焦点をややずらすことにより、日齢との識別が可能であった。